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What is Lyme Arthritis - also called Lyme Disease, & Chronic Lyme Arthritis ?

Lyme Arthritis is an epidemic, immune-mediated inflammatory disorder - usually begins with a characteristic skin lesion, ref. to as erythema chronicum migrans, - that may be followed weeks-to months later by neurologic or cardiac abnormalities, migratory polyarthritis, intermittent attacks of oligoarticular arthritis, or chronic arthritis in the knees.

The endemic areas for this disorder include the northeastern coast of the U.S., particularly southern Connecticut, southern Rhode Island, Cape Cod, southern New York and Long Island, New Jersey, Delaware and Maryland. It has also been reported in Northern Wisconsin in northern California and Oregon.

Lyme Arthritis or Lyme disease got its name from the small community of Lyme, in eastern Connecticut where

Lyme arthritis is thought to be caused by an infectious agent transmitted by I. dammini.

By a characteristic expanding skin lesion, ECM, patients may often be identified 1-3 weeks after exposure and before the onset of arthritis.

When the skin lesion is present, most patients have circulating immune complexes. At that time, those with high serum IgM levels, cryoglobulins containing IgM, and low IgG levels are the ones at risk of developing arthritis within months. Those with high IgG usually do not.

-the name - Erythema chronicum migrans.

ECM appears about 1-3 weeks after tick bite as an erythematous macule or papule. The borders of this lesion then expand to form a red ring as great as 20 to 30 cm in diameter, with central clearing. Occasionally secondary rings may form within the original one (erythema multiforme), sometimes expansion of the ring may not be accompanied by central clearing. The lesion which often itches, pricklies, or burns may be accompanied by fever, headache, vomiting, fatigue, and regional adenopathy.

It is of interest to note that the relatively voluminous European literature on ECM does not report or refer to arthritis - there is one possible exception - a report describing pains in a patient's ankle a few weeks after appearance of the ECM.

It also should be noted that the first case of ECM in the United States was reported in 1970, from the town of Redford in north central Wisconsin - so the

Even

A second passage to a third person was successful and produced the typical FCM rash - two weeks after inoculation!

Of particular interest to us - as well as to the studies currently done at CDC in Atlanta - is a 1948 paper by Lenhoff (Lenhoff C, 1948 - spirochetes in aetiologically obscure diseases Acta Derm Venereol Stockh 28; 295-324)

who reported on the presence of spirochetes in the FCM lesion. However, subsequent studies by others using darkfield and phase contrast microscopy failed to confirm Lenhoff's claim.

> In 1962, the French investigators Degos, Touraine and Abouelo reviewed the clinical histories of 7 FCM cases that occurred in France between 1958 and 1961. They submitted the sera of their patients to Paul Giroud (L'Inst. Pasteur, Paris) who examined them for antibodies to rickettsial antigens by his famous slide agglutination test. Giroud's results showed antibodies in titers up to 1:320 against R. prowazekii and up to 1:160 against R. mooseri and R. conorii in early

disease has been recognized before the outbreaks in Connecticut.

What are the factors that suggest that Lyme arthritis and ECM are tick-borne?

(1) - the geographic distribution of ECM and of Lyme arthritis coincide with the distribution of ixodid ticks, namely
I. ricinus in European countries
I. dammini along the northeastern coast and in Wisconsin and
I. pacificus in California and Oregon,

In many instances patients remembered having been bitten by ticks and in some instances patients provided the ticks for identification. Thus history of tick-bites prior to onset of the disorders are frequent.

(2) In a study carried out along the Connecticut river in Connecticut, it was found that the incidence of Lyme disease during 1977 was 2.8 cases per 1,000 residents on the east side of the river - whereas on the west side the incidence was only 0.1 cases per 1,000 residents. An accurate epidemiological survey revealed that the

population of *I. dammini* was much greater on the east compared to the west side of the river.

(3) The occurrence of cases in the summer and early fall, in the US at least coincided with activity seasons of *I. dammini*

(4) The incubation period of 1 to 3 weeks is relatively constant in both ECM & Lyme arthritis —

skills of ticks

There are areas such as in Scandinavia that report ECM cases but have no ticks; in those ~~areas~~ areas a mosquito vector has been suspected.

Alan Barbour

The causative agent(s) of ECM and Lyme arthritis are still unknown. Some investigators postulated a reaction to the tick bite - however the interval from the bite to the lesion - esp to 8 months seems too long for an allergic reaction to an ironfectious antigen. The most widely held view is that an infectious agent is involved: in fact German investigators succeeded to transmit the lesion among themselves by inoculating tissue from the edge of the lesion (Binder F., Doepfmer, R., Horstein O., 1958, Klin. Wochschr. 33: 727-728)

Let us now briefly discuss the biology of this medically important tick vector and refer to observations made by Carey, Kinsley and Main, Jan of the Department of Epidemiology and Public Health, Yale University School of Medicine at New Haven Conn. J. Med. Entomol. vol 17: 89-99, 1980 who studied this tick within the Lyme endemic areas of southern Connecticut.

I. dammini like the other members of the Ixodes ricinus complex has 3 developmental stages; the larva, the nymph, and the adult. Larval and nymphal ticks require a blood meal to reach the nymphal and adult stage, respectively. A blood meal is also required by the mated female tick before oviposition of eggs occurs. Male ticks do not ingest blood.

In 1978 according to Carey and associates larval I. dammini were most abundant in the late summer and adults in the spring and fall.

I. dammini was found to parasitize a large variety of mammals with the principle small mammal host for larvae

and nymphs being - the white-
footed mouse (Peromyscus leucopus)
and the Eastern chipmunk (Tamias
striatus). The principal host for the
adult was the white-tailed deer.
The immature stages of I. dammini
infest also cats, dogs, and - very
important - man!

White-tailed deer - have been reported
to be important host for all stages of
I. dammini (Piesman et al. 1979,
J. Med. Entomol. 15: 537-540)

unsuccessful. Also I should be stated that they have tested hundreds of patient sera for antibodies to tick-borne viral - other arboviral and bacterial agents with uniformly negative results

In 1988, Bob Philip and I tested a large series of Dr. Hess's sera by indirect immunofluorescence and microagglutination respectively against all available rickettsial antigens. The data were interpreted as being against a rickettsial etiology of Lyme disease.

Similarly, sera collected by Dr. Claes Kjellmark from ECM patients in Sweden had no significant titers when tested against the European members of the spotted fever group.

Last September, I unexpectedly received a shipment of adult I. dammini from my colleague Dr. Joyce Banach from the New York Department of Health at Stony Brook. In the past few years Joyce and I have collaborated on RMSF on Long Island. He more recently has been interested in the role of I. dammini as a vector of Babesia microti, the

causative agent of human Colorado in that area. Attached to the tick vial was a pencil-written note saying, "Collected from an area where several cases of Lyme arthritis have occurred. More ticks to follow, if you want to look at them – if not – don't. Jay."

Yes, we were interested in these ticks, particularly since previous examination of I. dammini from that area yielded specimens infected with spotted fever group rickettsiae. Attempts to isolate, however, had failed.

Thus the 21 ♂♂ and 23 ♀♀ were subjected to the hemolymph test. Although they were negative for rickettsiae, two females contained in their hemolymph masses (clumps) of a microfilaria.

✓✓✓✓✓✓ slides.

Although I am not a helminthologist a review of the literature on zoonotic filarial infections of North America suggests that the "beast" in question is a Dirofilaria.

Our slides have been forwarded for identification to the Department of Tropical Medicine and Public Health at the Tulane University.
~~until we receive final identification~~
~~In the meantime~~ let us realize that Dirofilaria is a nematode found in carnivores throughout the world mosquitoes and fleas are incriminated as the usual vectors.

In the U.S. veterinarians see Dirofilaria as heartworms in dogs and as subcutaneous infection in raccoons. About 50 human cases have so far been reported - most of these intensively enough - from New York, Long Island, Vermont, Massachusetts (Cape Cod), Florida and Wisconsin - i.e. within the distributional area of I. dammini.

Could it be that skin lock rather than mosquitoes or fleas is the vector to man?

After discovering the microfilariae, we decided to take a closer look at the remaining five ticks. Each tick was dissected for the preparation and examination of mean gut tissues including midgut salivary glands, Malpighian tubules, ganglion and genital systems.

Our first attention was directed towards a characterization of the tick's symbiotes.

Symbiotes as you must know are associated with every species of tick and occur in every tick specimen where they are usually limited to certain tissues such as the salivary Malpighian tubules and certain parts of the midgut. Their physiologic function and purpose are as yet unknown - best to the best of my knowledge they have ~~so far~~ never been linked to disease of a tick's natural or accidental host. Because of their absence from the tissues of the salivary glands, their transmission by tick can be ruled out.

The other rickettsia-like symbiotes occur either free in the cytoplasm or

as colonies in membrane-bound vacuoles.

✓✓✓ The symbiots of *T. dammini* - as illustrated in the next slides - are readily recognized as highly pleomorphic rickettsia-like organisms ranging from coccal to threadlike. They occur free in the cytoplasm where they produce massive infestations especially in the ovarial tissues.

In their fine structure they greatly differ from the symbiots of *Dermacentor*, for instance, in that

✓✓ a) they are not membrane-bound

b) there is no differentiation of their cytoplasm, and

c) they do not exhibit a multilamellar cell wall.

They resemble rickettsiae in their gram appearance and presence of a typical slime layer but differ from pathogenic rickettsiae by the absence of a microcapsular layer and the fact that the outer and inner leaflets of the cell wall are of the same thickness (2-3 nm).

Presently we are in the process of isolating these symbiotes as tissue cultures for immunologic and immunochemical characterization.

The results of preliminary FA stainings suggest that the T. dammini symbiotes have no antigenic relationship to the symbiotes of Dermacentor tick for instance or to the spotted fever or typhus group rickettsiae.

While examining the tissue smears of a series of 5 T. dammini ♀♀ I encountered yet another ♀ ♀ unit in the midgut of the second ♀ of that particular series — there it is - T. Spirochete!

To make certain that these organisms were alive, we prepared fresh preparations of midgut from the remains of the same tick and examined them under darkfield. Although many spirochetes were inactive, few exhibited the typical movement of BORRELIA. I shared this information with Jorge Benach who immediately

went with the field to collect and furnish additional ticks — and also with Alan Barbour who is collaborating with Dr. Hammer on the antigenic makeup of the relapsing fever agent, Borrelia hermsii.

As of today I have dissected 122 I. dammini (25 ♀♀ & 97 ♂♂) from the collection site in Long Island — 76 (12 ♀♀ & 64 ♂♂) were positive for spirochetes!

Interestingly there appears to be passed only in the ticks' digestive tract, particularly in the midgut where they are associated often in clumps — with the marcellar basal border of the gut epithelium. Spirochetes have never been observed in the hemolymph or in any other tissue of the ticks.

That we are dealing here with a Borrelia became apparent also from the following slide that illustrates the fine structure of this microorganism.

The first two slides are longitudinal sections showing the flagellum which consists of 6 to 8 fibrils (in the average) and which forms an integral part of the outer membrane. This is better seen in cross section that also illustrate the plasma-membrane (7-10 nm) surrounding a mottled cytoplasm with randomly distributed ribosomes.

The diameter of these organisms varies from .15 to .25 μ.

Of particular interest - and as yet unsolved - is the "blebbing" phenomenon - detectable even by conventional light and darkfield microscopy (a bleb or aneurism along the spirochete - as seen on previous slide).

Electron microscopy reveals that these blebs represent a bulging or aneurism of the outer cell membrane - forming a bleb that may contain numerous rather large granules. This phenomenon has been the subject

of many previous investigations by spirochetologists and has been interpreted as a "sporulation phase" in the developmental cycle of Borrelia - that usually multiply by transverse fission.

However, there are other investigators such as Pillot & Ryter (Ann. Institut Pasteur, Vol. 107, 1964) who state "spherical forms are only a degeneration product of the helicoidal elements. They do not constitute resistance forms nor elements of an obligatory cycle.

I am certain that we will address ourselves to this question in the near future, for Alan has been successful in isolating and maintaining in his Kelly's Medium the spirochete from a pool of tick midguts.

I should add here that inoculation of infected midgut suspensions and of heavily positive cultures into suckling and 21-day-old mice, also BALB/c RML white mice,

mice, meadow voles, guinea pigs, and rabbits did not result in detectable spirochetemias or illness.

√√ Finally, there was as yet another agent ~~in many tick smears~~ that I first considered to be of protozoan nature -

show slide -

Does anyone who has not heard of our work - recognize it?

Well, we had no difficulty in isolating this in Vero culture - when we recognized it as the developmental stage of a yeast - originating from the exoskeleton of the ticks.

Thus my friends, we are looking at at least 4 parasitic or microbial agents that are associated with I. dammini from a highly endemic area of Lyme disease.

Without the knowledge that Penicillin is highly effective in treating ECM and

Lyme disease, are called speculate that each of these agents could be involved in the etiology of these disorders. Panicoleum sensitivity, however, make the rickettsia-like symbiotes and particularly the spirochete prime layers for further investigations.

Such investigations were initiated a few days ago with sera collected by Jorge Benach of Lyme disease patients from Stony Island.

I am delighted to report that indirect fluorescence microscopy on either cultured or tick-associated spirochete gave very strong and specific staining reactions but was consistently negative when applied to the rickettsialike symbiotes.

These findings - as far as the spirochetes are concerned at least - were confirmed also by Alan Who applied the Western Blot Technique to show the relationship between the patients' antibodies and the spirochetal antigens.